*A REVIEW.........*

# Bioremediation technologies

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**ABSTRACT......** Bioremediation is an invaluable tool box for wider application in the realm of environmental protection. Bioremediation technologies have become attractive alternatives to conventional cleanup technologies due to relatively low capital costs and their inherently aesthetic nature. Bioremediation is generally considered to include natural attenuation (little or no human action), bio-stimulation or bio-augmentation, the deliberate addition of natural or engineered micro-organisms to accelerate the desired catalytic capabilities. The scope of environmental bioremediation extends to: Inorganics *viz.,* arsenic, mercury, chromium, fluoride, cyanide, abandoned mines, fly ash disposed sites, engineered phytotreatment technologies, biological permeable barriers and organics *viz.,* petroleum hydrocarbons, pesticides and explosives. A variety of plants, natural, transgenic and/ or associated with rhizosphere micro-organisms are extraordinarily active in these biological interventions and in cleaning up pollutants by removing or immobilizing them. Bioremediation approach is currently applied to contain contaminants in soil, groundwater, surface water and sediments including air. Thus, Bioremediation is emerging as an effective, environment friendly and innovative technology for treatment of a wide variety of contaminants in water and soil.

**KEY WORDS......** Bioremediation, Bio stimulation, Bio augmentation, Wetland construction

Rapid industrialization and urbanization over the past many decades has resulted in contamination of all the components of the environment that is the air, the water, the soils. The contamination of the environment has also occurred through a variety of industrial operations like fugitive emissions, accidental spills and leaks, discharge of effluents or dumping of waste. Thus, the challenge today is to develop technologies that consume fewer resources, incorporate recycling and reuse of components thereby reducing the production of wastes while maintaining/improving the efficiency. Therefore, there is a need to develop a wide variety of physico-chemical and biological techniques that can remediate the hazardous contaminants from the environment without causing further damage. The conventional techniques of incineration/land-fills etc. basically transfer pollutants from one medium to another, are expensive and energy demanding. Further, these techniques are often inefficient for handling voluminous effluents containing complexing organic matter and low concentration of contaminants. Biotechnological approaches that are designed to cover such niches have, therefore, received great deal of attention in the recent years.

Bioremediation is emerging as an attractive,

potential, effective and innovative technology for treatment of a wide variety of contaminants. Bioremediation is the process of using micro-organisms to transform hazardous chemical compounds in a contaminated soil, sediment to non-hazardous end products. Almost all-organic compounds and some of the inorganic compounds can be degraded biologically if sufficient time and proper physical and chemical conditions are provided.

Bioremediation has been successfully applied for clean up of soil, surface water, groundwater, sediments and ecosystem restoration. The present day bioremediation technologies are based on the processes and potentials of almost all types of life forms, *viz.,* plants (phytoremediation), micro-organisms (microbial remediation), rhizoremediation (plant and microbe interaction), phycoremediation (algae) and zooremediation (animals) (Srivastava, 2015). Thus, bioremediation, is the use of biological interventions of biodiversity for mitigation (and wherever possible, complete elimination) of the noxious effects caused by environmental pollutants in a given site. It contribute significantly to the fate of hazardous waste and can be used to remove these unwanted compounds from the biosphere (1) (Ma *et al.*, 2011 and Schroeder and Schwitzguebel, 2004).

**Objectives of bioremediation :**

The main objectives of bioremediation can be 1) Enhancing the rate and extent of biodegradation of the pollutants in consideration 2) Utilizing or developing micro-organisms that is capable of surviving the toxic effects of the pollutants and 3) utilizing the micro-organisms in such a way that the products of the degradation process are not toxic.

**Bioremediation strategies :**

Two basic methods are available for obtaining the micro-organisms necessary to initiate bioremediation :

*Bio-stimulation* :

It involves the injection of the necessary nutrients to stimulate the growth of indigenous micro-organisms. For example, addition of nutrients, oxygen, or other electron donors and acceptors. These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants.

All of them will need nitrogen, phosphorus, and carbon. Removal of inorganic materials by micro-organisms may be achieved when the organic compounds are utilized as electron donors by the micro-organism. Fertilizers and growth supplements are common stimulants. Presence of small amount of pollutant can also act as stimulant by turning on the operons for bioremediation enzymes. Biostimulation can be done *in situ* or *ex situ* (Andreoni *et al.*, 1998).

*Bioaugmentation* :

Bioaugmentation is the introduction of micro-organisms with specific catabolic abilities into the contaminated environment in order to supplement the indigenous population and to speedup or enable the degradation of pollutants (Louisa, 2010). It involves the addition of pollutant-degrading micro-organisms of both type *i.e.* indigenous or exogenous (natural/exotic/acclimatized/genetically engineered) to augment the biodegradative capacity of indigenous microbial populations. Sometimes micro-organisms from the remediation site are collected, separately cultured, and returned to the site as a means of rapidly increasing the micro-organism population at the site. Usually an attempt is made to isolate and accelerate the growth of the population of natural micro-organisms that preferentially feed on the contaminants at the site. In some situations different micro-organisms may be added at different stages of the remediation process because the contaminants change in abundance as the degradation proceeds (Da Silva and Alvarez, 2004 and Jitnuyanont *et al.,* 2001). Enhanced anaerobic biodegradation of benzene-toluene-ethylbenzene-xylene-ethanol mixtures in bioaugmented aquifer columns, appl environ microbiol 70, 4720–4726. Two factors limit the use of added microbial cultures in a land treatment unit: 1) nonindigenous cultures rarely compete well enough with an indigenous population to develop and sustain useful population levels and 2) most soils with long-term exposure to biodegradable waste have indigenous micro-organisms that are effective degrades if the land treatment unit is well managed.

**Bioremediation technologies :**

There are different treatment technologies under bioremediation and before we begin exploring the basics

of bioremediation, let us get acquainted with these terms.

## Intrinsic bioremediation :

This is a process whereby the natural microflora and environmental conditions exist for natural attenuation of a pollutant to safe levels within acceptable time frame. Here natural subsurface processes such as dilution, volatilization, biodegradation, adsorption, and chemical reactions with subsurface materials are allowed to reduce contaminant concentrations to acceptable levels. This requires no intervention but just monitoring of the natural process of biodegradation. However, it should not be perceived as "no action" plan as long term monitoring must be conducted throughout the process to confirm that degradation is proceeding at rates consistent with meeting cleanup objectives.

Compared with other remediation technologies, natural attenuation has several advantages such as less generation or transfer of remediation wastes; less intrusive (as few surface structures are required) and may be applied to all or part of a given site, depending on site conditions and cleanup objectives. Further it may be used in conjunction with, or as a follow-up to, other (active) remedial measures and the overall cost will likely be lower than active remediation.

## *In situ* bioremediation :

*In situ* bioremediation techniques are defined as those that are applied to soil and groundwater at the site/ in the same place affected by pollution (U.S. EPA/625/ K-96/001 and U.S. EPA/540/2-90/002). These techniques are generally the most desirable options due to lower cost and less disturbance since they provide the treatment in place avoiding excavation and transport of contaminants (Gallego *et al.*, 2001 and Mishra *et al.*, 1999 and 2001). *In situ* treatment is limited by the depth of the soil that can be effectively treated. In many soils effective oxygen diffusion for desirable rates of bioremediation extend to a range of only a few cm to about 30 cm into the soil, although depths of 60 cm and greater have been effectively treated in some cases.

## *Ex situ* bioremediation :

*Ex situ* techniques are those that are applied to soil and groundwater at the site which has been removed from the site to a different place to accelerate biocatalysis. These techniques involve the excavation or removal of contaminated soil and water from ground.

The most important land treatments are:

## Bioventing :

It is the most common in situ treatment and involves oxygen and nutrients by circulating aqueous solutions through contaminated soils to stimulate naturally occurring bacteria to degrade organic contaminants. Thus, bioventing may complement biostimulation as well as bioaugmentation. It is a promising technology that stimulates the natural in situ biodegradation of any aerobically degradable compounds in soil by providing oxygen to existing soil micro-organisms. This technique includes conditions such as the infiltration of water-containing nutrients and oxygen or other electron acceptors for groundwater treatment. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface.

Bioventing techniques have been successfully used to remediate soils contaminated by petroleum hydrocarbons, non-chlorinated solvents, some pesticides, wood preservatives, and other organic chemicals. This technique shows considerable promise of stabilizing or removing inorganics from soil as it can induce changes in the valence state of inorganics and cause adsorption, uptake, accumulation, and concentration of inorganics in micro or macro-organisms. However, several factors may limit the applicability and effectiveness of the process for example highly saturated soils, extremely low moisture content or low permeability soils negatively affect the bioventing performance.

## Biosparging :

Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system.

Landfarming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous

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biodegradative micro-organisms and facilitate their aerobic degradation of contaminants. Different conditions that are controlled during land farming are moisture content (usually by irrigation or spraying), aeration (by tilling the soil with a predetermined frequency), pH (buffered near neutral pH by adding crushed limestone or agricultural lime) and other amendments (e.g., Soil bulking agents, nutrients, etc.). In general, the practice is limited to the treatment of superficial 10–35 cm of soil. Since landfarming has the potential to reduce monitoring and maintenance costs, as well as clean-up liabilities, it has received much attention as a disposal alternative.

It may be done *in situ* or in a treatment cell and has been successfully used to remove large petroleum spills, wood-preserving wastes (PCP and creosote), coke wastes, and certain pesticides in the soil (El-Nawawy *et al.,* 1995). The large requirement of space, proper management of leachates and prevention of volatile gases are some of the limitations associated with landfarming.

Composting is a technique that involves combining contaminated soil with non-hazardous organic amendants such as manure or agricultural wastes. It is aerobic, thermophilic and controlled biological process by which organic contaminants (e.g., PAHs) are converted by micro-organisms to safe, stabilized by products. Typically, thermophilic conditions (54 to 65°C) must be maintained to properly compost soil contaminated with hazardous organic contaminants and in most cases, this is achieved by the use of indigenous micro-organisms treatment. Soils are excavated and mixed with bulking agents and organic amendments, such as wood chips, animal, and vegetative wastes etc. to enhance the porosity of the mixture to be decomposed. Maximum degradation efficiency is achieved through maintaining aeration and moisture as necessary, and closely monitoring moisture content, and temperature. Basically three different process designs are used in composting: 1. Aerated static pile composting where compost is formed into piles and aerated with blowers or vacuum pumps. 2. Mechanically agitated in-vessel composting where compost is placed in a reactor vessel, mixed and aerated. 3. Windrow composting where compost is placed in long piles known as windrows and periodically mixed with mobile equipment .Windrow composting is usually considered to be the most cost-effective composting alternative but it may also have the highest fugitive emissions. Pilot and full-scale projects have demonstrated that aerobic, thermophilic composting is able to reduce the concentration of explosives (TNT, RDX, and HMX), ammonium picrate (or yellow-D), and associated toxicity to acceptable levels and is also applicable to PAH-contaminated soil. The substantial requirement of space and aeration coupled with the need for excavation of contaminated soil limit the application of composting. If VOC (volatile organic compounds) or SVOC (semi-volatile organic compounds) contaminants are present in soils, off-gas control may be required. Lastly, this method cannot treat metals and due to addition of amendments ultimately leads to volumetric increase in the amount.

Biopiles are a hybrid of landfarming and composting (Fahnestock *et al.,* 1998). Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of landfarming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic micro-organisms.

**Bioreactors :**

A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous micro-organisms) capable of degrading target contaminants. Slurry reactors or aqueous reactors are used for *ex situ* treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment, sludge) or water through an engineered containment system. A slurry bioreactor may be defined as a containment vessel and apparatus used to createa three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil bound and water-soluble pollutants as a water slurry of the contaminated soil and biomass (usually indigenous micro-organisms) capable of degrading target contaminants. In general, the rate and extent of biodegradation are greater in a bioreactor system than *in situ* or in solid-phase systems because the contained environment is more manageable and hence, more controllable and predictable (Shim *et al*., 2002). Despite the advantages of reactor systems,

there are some disadvantages. The contaminated soil requires pre treatment (e.g., excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (e.g., vacuum extraction) before being placed in a bioreactor.

### Biofilters :

Use of microbial stripping columns (containing micro-organism enriched compost/soil) is to treat organic gases (volatile organic compounds).

### Wetland construction :

Constructed wetlands are the result of human skill and technology integrating geology, hydrology and biology. Two kinds of wetlands are in the service of mankind: a) Natural wetlands - used for wastewater treatment for centuries; b) Constructed wetlands – effective in treating organic matter, nitrogen, phosphorus, decreasing the concentrations of trace metals and organic chemicals (Kadlec and Knight, 1996).

Wetland plants have the ability to either take up oxygen from the air or use photosynthetic oxygen and translocate the oxygen to the roots and into the rhizosphere. Thereby, they will increase the redox potential and thus, also decrease the pH and increase the release of metals. Aquatic plants can tolerate a very low pH, which can be necessary when treating AMD. Carex rostrata, Phragmites australis, Typha angustifolia, T. latifolia, have been found growing under field conditions in pH as low as 2-4.4. The submerged aquatic macrophytes have very thin cuticles and, therefore, readily take up metals from water through the entire surface. Aquatic macrophytes e.g. *Typha latifolia*, *Eichhornia crassipes*, *Ipomea* sp. *Lemna minor*, *Polygonum* sp., *Alternanthera philoxeroides*, *Phragmites* sp. have paramount significance in bioremediation. These are being used in water quality assessment and also as fast track botanical cleanup crews (Prasad, 2007 and Prasad *et al.*, 2006). They are important in nutrient cycling, controlling water quality, sediment stabilization and provision of habitat for a host of aquatic organisms.

Constructed wetlands are being designed for the treatment of municipal waste waters in developed nations. Due to the cost-effectiveness in the treatment of non-point source pollution, use of constructed wetlands is rapidly spreading in developed nations. However, in tropical nations due to water scarcity and high surface evapo-transpiration, the constructed wetlands for treatment of waste waters is not gaining importance.

### Advantages :

All the current technologies that are available for treatment of waste have lots of limitations. Most of them are not cost effective and are inappropriate for the in situ treatment. Some of them are not effective in treating a complex array of different pollutants. Biological treatment appears to offer solution to these limitations. The basic advantage of bioremediation are 1) Biodegradation is a "natural" process in which naturally occurring micro-organisms are used for treatment of wastes. 2) The residues or the by-products of biological processes ($CO_2$, $H_2O$) are usually geochemically cycled in the environment as harmless products. 3). Micro-organisms such as bacteria and fungi have a wide range of abilities to metabolize different chemicals. 4) the technologies are developed to utilize and improve native micro-organisms that have been demonstrated to degrade the pollutants on site. In this case, adding nutrients or other amendments to the site can accelerate the rate of microbial activity. In other cases, micro-organism known to metabolize the pollutants can be introduced and supplemented to improve biodegradation. 5) For in situ treatment of soils, sludge and ground water, bioremediation is less expensive and less disruptive than options frequently used for treatment, such as excavation followed by incineration or land filling. Today, bioremediation is widely applied in the treatment of contaminated water, soil, sludge, and sediments. Bioremediation is the best method for remediation of the long chain molecular organic compounds, hazardous waste and toxicity chemical.

### Conclusion :

Due to rapid economic development, in recent times, environmental pollution and resources depletion problems have become major issues affecting the sustainable social and economic development. The balance between the economic growth and environmental/ecological protection has gradually become one of the key issues that should be taken into consideration by government officials, policy makers, company managers, scientists and engineers. Bioremediation is cost effective, solar driven, faster

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than natural attenuation, high public acceptance including enhancement of aesthetics, and generates less secondary wastes with fewer air and water emissions. The recent interdisciplinary approach of environmental problem solving through combination of biotechnology, microbiology, genetic engineering on the sphere of ecological practices has given rise to promising research and application of bioremediation tools. Since bioremediation is based on natural attenuation the public considers it more acceptable than other technologies. As these technologies are quite safe compared to the application of chemical compounds, there is great potential for this technology. However, for optimizing the maximal benefit sustainability of such technologies, requires an effective policy directives supported by social perception and must necessarily incorporate the application of environmental impact assessments, intellectual property rights and cost-benefit analysis for commercial viability. Thus Bioremediation is emerging as an effective, environment friendly and innovative technology for treatment of a wide variety of contaminants in water and soil.

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